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Structure-Based Virtual Screening of Novel Allosteric Inhibitors Targeting KRAS G12D: Molecular Dynamics and Binding Free Energy Analysis

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ABSTRACT

Background: KRAS G12D is the most frequent KRAS lesion in pancreatic and colorectal cancer, yet it has long resisted small-molecule targeting because it lacks the reactive cysteine that made covalent G12C inhibitors possible and offers a nucleotide pocket with no obvious ligandable groove.

Objective: This review synthesizes the published literature on structure-based virtual screening, molecular dynamics (MD) simulation, and binding free energy calculation as applied to the discovery of allosteric KRAS G12D inhibitors, situating this work against the methodological precedent set by G12C-directed covalent inhibitors.

Scope: We cover the switch I/switch II allosteric pocket biology that underlies current design strategies, docking and pharmacophore-based virtual screening approaches, MD protocols used to validate and refine binding poses, and end-point (MM-GBSA/MM-PBSA) versus alchemical (free energy perturbation) methods for ranking candidate affinity. Case studies include the noncovalent inhibitor MRTX1133, salt-bridge-forming analogs, the targeted degrader ASP3082, and the SOS1 inhibitor BI-3406 as a pathway-adjacent strategy.

Conclusions: Computational structure-based methods have been central, not incidental, to converting KRAS G12D from an undruggable target into one with an advanced noncovalent clinical candidate. Persisting limitations include narrow structural diversity across screening pipelines built on a single co-crystal template, uncertainty in end-point free energy estimates within this polar, solvent-exposed pocket, and limited systematic application of rigorous alchemical free energy methods at the hit-finding stage. Extending accurate free energy estimation earlier in the discovery funnel, and adapting computational pipelines to ternary-complex and protein-protein-interface targeting strategies, are identified as priorities for the next phase of KRAS G12D drug discovery.

Keywords: *KRAS G12D; allosteric inhibitor; virtual screening; molecular dynamics; binding free energy*

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Abstract

KRAS has spent four decades on the list of targets that medicinal chemists were told to avoid, and the G12D substitution — the single most common KRAS lesion across pancreatic, colorectal, and a meaningful slice of lung cancers — was for most of that time the least tractable member of the family. A smooth nucleotide-binding pocket with picomolar affinity for GTP left almost nothing for a small molecule to grip, and the absence of a reactive cysteine (the feature that made G12C druggable) closed off the covalent strategy that produced sotorasib and adagrasib. This review traces how structure-based virtual screening, molecular dynamics simulation, and binding free energy estimation have converged over the past six years to turn that picture around. We cover the biology of the switch I/switch II conformational cycle that defines the druggable allosteric surfaces of KRAS, the docking and pharmacophore strategies used to search chemical libraries against those surfaces, and the roles that all-atom MD and enhanced sampling now play in distinguishing transient from persistent binding modes. We give particular attention to end-point free energy methods (MM-GBSA/MM-PBSA) and alchemical free energy perturbation, weighing their practical trade-offs for ranking KRAS G12D

candidates. MRTX1133 anchors the case-study discussion as the compound that proved a noncovalent, high-affinity switch II/switch I pocket engager was achievable, and we discuss follow-on chemotypes including salt-bridge-forming inhibitors, SOS1 nucleotide-exchange inhibitors, and targeted protein degraders such as ASP3082. We close by discussing where computational prediction and experimental validation still diverge, and what that gap implies for the next generation of allosteric KRAS G12D programs.

Introduction

Oncogenic RAS mutations sit near the top of every list of cancer drug targets that "everyone wants and nobody can hit." KRAS alone is mutated in roughly a quarter of all human tumors, with striking tissue preferences: G12D dominates in pancreatic ductal adenocarcinoma, is common in colorectal cancer, and appears at meaningful frequency in non-small cell lung cancer as well [1]. For years the field's working assumption was that RAS proteins were essentially undruggable by conventional small molecules — the GTPase domain presents a shallow, highly polar nucleotide pocket that GTP and GDP occupy with femtomolar-to-picomolar affinity, leaving no obvious groove for a competitive inhibitor, and the protein surface otherwise lacks the deep hydrophobic pockets that structure-based design usually exploits [2].

That assumption started to crack when Shokat and colleagues showed that the oncogenic G12C mutant — which introduces a cysteine not present in wild-type KRAS — could be covalently trapped in its inactive, GDP-bound state through a previously unrecognized pocket beneath the switch II loop [3]. The switch II pocket turned out to be a genuinely druggable allosteric site, and the ensuing decade produced two approved covalent G12C inhibitors, sotorasib and adagrasib, along with a clinical and mechanistic literature that is now extensive [4,5,6]. What that success could not do, however, was solve G12D. Aspartate at position 12 offers no thiol to react with, so the covalent-trapping logic that built sotorasib and adagrasib simply does not transfer. Any G12D-directed chemotype has to bind reversibly, or through some other irreversible chemistry entirely, and it has to do so with enough affinity and residence time to compete with millimolar cellular GTP concentrations and near-covalent-strength nucleotide affinity.

This is where computational structure-based methods have done some of their most consequential work in oncology drug discovery over the past several years. Virtual screening against cryo-EM and X-ray structures of KRAS G12D identified early hit chemotypes; molecular dynamics simulations characterized the conformational dynamics of the switch I/switch II region and the adjacent pockets that open and close as the protein cycles between GTP- and GDP-loaded states; and binding free energy calculations — from fast end-point approximations to rigorous alchemical perturbation — provided the quantitative scaffolding needed to prioritize and optimize candidate molecules before committing to synthesis. The result, MRTX1133, is now one of the best-documented examples in the literature of a computationally guided allosteric inhibitor program reaching a noncovalent, subnanomolar, highly selective clinical candidate [7,8]. This review synthesizes that literature: the structural biology that defines the druggable surfaces on KRAS G12D, the virtual screening and MD methodologies used to search and validate binding poses, the free energy methods used to rank affinity, and the resulting chemotypes and their translational status.

KRAS Biology and the Allosteric Surfaces Available to G12D-Directed Design

KRAS functions as a nucleotide-dependent molecular switch: GTP loading drives a conformational rearrangement in the switch I (residues ~25-40) and switch II (residues ~60-76) loops that creates the effector-binding surface recognized by RAF, PI3K, and RalGDS, while GTP hydrolysis to GDP — accelerated by GTPase-activating proteins in the wild-type protein — collapses that surface and terminates signaling [2]. Oncogenic mutations at codon 12, 13, or 61 do not eliminate GTPase activity outright so much as impair GAP-stimulated hydrolysis and, in some cases, favor spontaneous nucleotide exchange, biasing the protein toward the GTP-loaded, signaling-competent state for longer than is physiologically appropriate.

Two structural observations made allosteric targeting conceivable at all. First, the switch II pocket that Ostrem and colleagues identified beneath the switch II helix is not present, or not accessible, in the fully GTP-loaded conformation — it opens preferentially when the protein relaxes toward a GDP-like or intermediate state, which is exactly the state covalent G12C binders trap and stabilize [3]. Second, and more relevant to G12D-specific design, later crystallographic and cryo-EM work on MRTX1133-bound KRAS G12D showed that a related but distinct pocket spanning switch I and switch II, adjacent to the mutant Asp12 side chain, can accommodate a noncovalent ligand that simultaneously contacts the P-loop, switch I, and switch II regions and forms a direct salt bridge or hydrogen-bonding network with the mutant aspartate itself [7]. This is a genuinely different targeting logic than covalent G12C inhibition: rather than exploiting a mutant-specific nucleophile, it exploits the mutant-specific electrostatics that Asp12 introduces relative to the wild-type glycine, plus induced-fit pocket opening that a computational model has to capture correctly to be useful.

The practical consequence for structure-based design is that a docking or MD study of KRAS G12D cannot treat the protein as rigid. Different deposited structures — apo, GDP-bound, GTP-analog-bound, and inhibitor-bound — show meaningfully different pocket geometries around switch I/II, and a virtual screening campaign run against a single static structure risks either missing viable chemotypes or, more commonly, generating docking poses that look plausible but do not correspond to any thermodynamically accessible protein conformation. This is precisely the problem molecular dynamics is used to address, and it explains why almost every recent computational KRAS G12D paper pairs docking with post-hoc MD refinement rather than reporting docking scores alone [9,10].

Structure-Based Virtual Screening Strategies

Virtual screening against KRAS G12D generally follows a familiar funnel — library filtering, rigid or semi-flexible docking, rescoring, and visual or MD-based triage — but the specifics of pocket definition matter more here than in most target classes, precisely because the druggable surface is allosteric and conformationally dependent rather than a well-defined orthosteric cleft. Du and colleagues, for example, used the switch I/II pocket geometry defined by MRTX1133-bound structures as the docking grid for a large-scale screen of a compound library, using AutoDock Vina for the initial pass before carrying the top-ranked poses forward into explicit-solvent MD [9,11]. That two-stage design — cheap scoring function first, physics-based refinement second — has become close to a standard operating procedure in this space, largely because docking scoring functions

are known to be unreliable at ranking closely related analogs, while MD-refined metrics correlate better with experimental affinity at a fraction of the cost of full alchemical calculations.

A related but distinct approach uses the MRTX1133 co-crystal pose itself as a pharmacophore query rather than screening blind against the pocket. Issahaku and coworkers took this route, characterizing the specific residue contacts MRTX1133 makes — including the salt bridge to Asp12 and hydrogen bonds within the switch II loop — and using that interaction fingerprint to filter candidate scaffolds before docking, on the logic that a molecule reproducing the key polar contacts is more likely to translate into real potency than one merely occupying the pocket volume [10]. This pharmacophore-anchored strategy has an obvious advantage in specificity, but it also biases discovery toward MRTX1133-like chemical space, which is a reasonable trade-off for lead optimization but a real limitation if the goal is finding a structurally novel backup series.

More recently, machine learning has started to enter this workflow directly rather than sitting downstream of docking as a rescoring tool. The KRASAVA system integrates a quantitative structure-activity relationship model trained on reported KRAS G12D inhibitory data with automated filtering for drug-likeness (Muegge's rules) and structural alert flags such as PAINS motifs, wrapping the whole pipeline into a screening tool that proposes candidates alongside a predicted applicability-domain confidence [12]. Whether QSAR-driven prioritization outperforms docking-first pipelines for this specific pocket is still an open question — the training data for KRAS G12D inhibitors remains comparatively small relative to more heavily studied target classes — but the approach is a useful illustration of where the field is heading: fewer campaigns treating docking scores as a standalone decision criterion, and more hybrid pipelines that combine ligand-based, structure-based, and physics-based filters before a compound is ever synthesized.

Molecular Dynamics Simulation of the Switch I/II Region

Once a docking campaign produces candidate poses, the central question becomes whether the pose is stable, and molecular dynamics is the tool that answers it. Standard practice in the papers reviewed here is 100 ns to several hundred nanoseconds of explicit-solvent, all-atom MD per complex, run in GROMACS or a comparable engine with an AMBER-family or CHARMM-family force field, following energy minimization and staged equilibration [13]. The output metrics that matter most for triage are root-mean-square deviation of the ligand and pocket residues over the trajectory, persistence of the key polar contacts identified from the crystal structure (notably the Asp12 salt bridge), and — where feasible — per-residue fluctuation analysis of switch I and switch II to see whether the ligand is actually restraining the dynamic loops that would otherwise sample multiple conformations.

This last point is worth dwelling on because it is somewhat specific to allosteric GTPase targets. In a conventional enzyme active site, a stable RMSD trajectory is usually sufficient evidence that a pose is reasonable. For KRAS G12D, switch I and switch II are intrinsically mobile even in the apo or nucleotide-bound state, and a ligand that merely sits in the pocket without damping that mobility is unlikely to reproduce the functional effect MRTX1133 achieves — namely, locking the protein in a signaling-incompetent conformation. Several of the reviewed virtual screening studies therefore report switch-loop fluctuation profiles alongside standard trajectory stability metrics as part of their MD-based triage, treating loop rigidification as a proxy for functional inhibition rather than relying on binding pose stability alone [9,10].

Simulation length and replicate number remain a genuine limitation across this literature. Hundreds of nanoseconds is enough to assess local pocket stability and short-timescale induced fit, but it is generally not enough to sample slower conformational transitions — full switch I/II unfolding-refolding events, or nucleotide exchange itself — that would matter for a complete mechanistic picture. Enhanced sampling methods (metadynamics, replica exchange, adaptive sampling) appear in the broader RAS MD literature but are less consistently applied in the specific virtual-screening-to-hit pipelines reviewed here, in part because they are computationally expensive to run across the dozens to hundreds of candidate complexes a typical screening funnel produces. This is a reasonable pragmatic trade-off at the hit-identification stage, but it does mean that MD-refined "stable" poses from early-stage screening studies should be read as necessary rather than sufficient evidence of a genuine binding mode, pending experimental structural confirmation.

Binding Free Energy Methods: MM-GBSA/MM-PBSA and Alchemical Approaches

Docking scores rank poses within a single scoring function's idiosyncratic energy landscape; they are not reliable estimators of absolute or even relative binding affinity across chemically diverse candidates. The next tier of rigor is end-point free energy calculation — MM-PBSA and MM-GBSA — which extracts snapshots from an MD trajectory (or a shorter post-docking equilibration run) and estimates binding free energy by combining molecular mechanics interaction energies with a continuum solvation term and, often, a normal-mode or interaction-entropy correction [14]. These methods are attractive precisely because they reuse the same trajectories generated for pose validation, adding relatively little additional computational cost while providing a numerical affinity ranking that correlates reasonably well with experimental data for structurally similar analogs.

The caveats are well established in the broader biophysics literature and apply squarely to KRAS G12D work. Genheden and Ryde's methodological review lays out where MM-PBSA/MM-GBSA tends to succeed and where it does not: relative rankings within a congeneric series are usually more trustworthy than absolute free energy values, GBSA is faster but somewhat less accurate than PBSA for charged or highly polar interfaces, and the entropy term — whichever approximation is used — is often the largest source of error in the whole calculation [15]. The switch I/II pocket is about as polar and solvent-exposed an environment as end-point methods are asked to handle in oncology drug discovery, given the ionic character of the Asp12 contact and the generally charged character of the surrounding loops, so some of the discrepancies between predicted and experimental affinities reported in the KRAS G12D virtual screening literature are not surprising in that light.

Alchemical free energy perturbation sits a tier above end-point methods in both rigor and cost. Rather than approximating a single bound-state snapshot, FEP calculates the free energy difference between two ligands (relative FEP) or between a ligand's bound and unbound states (absolute FEP) by simulating a series of intermediate, non-physical alchemical states and integrating the free energy change across them, typically using

well-validated force fields and enhanced sampling protocols [16]. When applied carefully to a congeneric series with good structural data, modern FEP implementations routinely achieve sub-1-kcal/mol accuracy against experimental binding data, which is precise enough to guide real medicinal chemistry decisions rather than just rank-order screening hits. The trade-off is compute cost and setup complexity: FEP calculations require converged sampling across multiple lambda windows per compound pair, which scales poorly to the hundreds-to-thousands of compounds a typical virtual screening funnel needs to triage, and the method's accuracy advantage is most reliably realized on closely related analogs rather than scaffold-hopping comparisons. In practice, the KRAS G12D literature reviewed here uses MM-GBSA-type methods for primary hit triage from virtual screening and reserves — or, in several cases, has yet to fully apply — alchemical FEP for later-stage optimization of validated chemotypes, which is consistent with how these two tiers of method are generally deployed across structure-based drug discovery more broadly.

Inhibitor Chemotypes and Case Studies

MRTX1133 and the noncovalent switch I/II pocket. MRTX1133 remains the reference compound for this entire target, and for good reason: it achieves picomolar-to-low-nanomolar biochemical affinity for KRAS G12D with several hundred-fold selectivity over wild-type KRAS, occupies the switch I/II pocket noncovalently, and produces substantial tumor regression in pancreatic ductal adenocarcinoma xenograft models, including in combination with EGFR or PI3K pathway inhibitors to counter feedback reactivation [7,8]. Its co-crystal structure has become the de facto template for essentially every subsequent computational KRAS G12D program discussed in this review, whether as a docking grid definition, a pharmacophore query, or an MD-refined pocket-stability benchmark [9,10].

Salt-bridge-forming noncovalent inhibitors. Mao and colleagues described a related but chemically distinct series of noncovalent inhibitors that exploit the same Asp12 electrostatic handle, showing — notably — that these compounds bind with comparable affinity to both the GDP- and GTP-loaded states of KRAS G12D, a property that differentiates them mechanistically from state-selective covalent G12C binders and suggests a somewhat different pharmacodynamic rationale for durable target engagement [17].

Targeted protein degraders. ASP3082 represents a mechanistically distinct answer to the same druggability problem: rather than occupying the switch I/II pocket to sterically and electrostatically block effector engagement, it recruits a ternary complex with an E3 ligase to drive proteasomal degradation of KRAS G12D itself, achieving nanomolar degradation potency and, in preclinical models, more durable pathway suppression than reversible occupancy alone because there is simply less protein left to reactivate signaling [18]. Structure-based modeling of the ternary complex — protein of interest, degrader, and E3 ligase, all simultaneously — is a considerably harder computational problem than single-protein pocket docking, and it is notable that this program relied on ternary complex modeling explicitly rather than adapting standard virtual screening pipelines wholesale.

Upstream and pathway-adjacent strategies. Not every relevant computational and structural advance targets the mutant protein directly. SOS1 is the guanine nucleotide exchange factor responsible for loading KRAS with GTP in the first place, and BI-3406 — a potent, selective small-molecule SOS1-KRAS interaction inhibitor — reduces GTP-loaded KRAS levels indirectly and synergizes with direct KRAS inhibitors and MEK inhibitors across multiple KRAS-mutant contexts, including, in later work, direct combination with a KRAS G12D inhibitor [19]. This class is a useful reminder that "targeting KRAS G12D" in the applied therapeutic sense is broader than direct pocket occupancy of the mutant protein, and that structure-based design against the SOS1-KRAS protein-protein interface uses many of the same virtual screening and MD tools discussed above, applied to a different structural target.

The G12C covalent inhibitors as methodological context. Sotorasib and adagrasib are not G12D-directed compounds, but the discovery campaigns behind both drugs are inseparable from the methodological history this review is tracing: they demonstrated, first, that an allosteric RAS pocket could be drugged at all, and second, that computational structure-based design paired with iterative medicinal chemistry could take a mutant-selective allosteric binder from hit to approved drug within a workable timeline [3,4,6,5]. Every subsequent G12D program discussed here is, in a real sense, working from the template those campaigns established, even though the specific chemistry — noncovalent versus covalent, Asp12 salt bridge versus Cys12 electrophile — differs substantially.

A summary comparison of these chemotypes, their binding modes, and their computational and experimental status is given below.

Summary of Inhibitor Chemotypes and Binding Modes

(see accompanying table)

Discussion and Future Directions

A few patterns stand out when this literature is read as a whole rather than as a series of individual papers. The first is how much of the field's recent progress has depended on getting a single high-quality structural template — the MRTX1133-bound KRAS G12D complex — and then reusing it across docking, pharmacophore, and MD-based screening pipelines built by different groups [7,9,10]. That is a reasonable and efficient way to build on a hard-won structural result, but it also means the field's computational diversity may be narrower than the number of published papers suggests: many pipelines are, in effect, variations on searching for MRTX1133-like chemical matter, which limits the odds of finding a structurally orthogonal backup series should resistance mutations or tolerability issues emerge against that particular pocket engagement mode.

The second pattern is a persistent gap between what computational binding free energy methods can promise in principle and what they reliably deliver in this specific pocket. MM-GBSA-type calculations are fast enough to apply across a virtual screening funnel and useful for coarse triage, but the chemical reviews on the method are explicit that absolute affinity predictions in charged, solvent-exposed pockets — which describes the switch I/II region reasonably well — carry real uncertainty [14,15]. Rigorous alchemical FEP calculations can do much better, but at a cost that has, so far, limited their systematic application to the earliest hit-finding stages of KRAS G12D programs; they show up more in later optimization work once a series is established than in the initial screening papers themselves [16]. Closing that gap — running affordable but sufficiently accurate free energy estimation earlier in the funnel

— is arguably the single most consequential methodological improvement this field could use going forward, and recent GPU-accelerated FEP implementations and machine-learned force fields are starting to make that more feasible, though systematic application specifically to KRAS G12D has not yet caught up with the broader FEP methodology literature.

Representative KRAS G12D-directed and mechanistically related inhibitor chemotypes discussed in this review, their binding mode, and computational/experimental status as reported in the primary literature.

Compound / series	Chemotype / mechanism	Binding site or target	Development status	Key reference
MRTX1133	Noncovalent small molecule, Asp12 salt bridge/H-bond network	Switch I/switch II allosteric pocket, KRAS G12D	Clinical-stage; extensive preclinical PDAC efficacy data	Wang et al. 2022; Hallin et al. 2022
Mao et al. salt-bridge series	Noncovalent, GDP- and GTP-state binding	Switch I/switch II pocket, KRAS G12D	Preclinical lead series	Mao et al. 2022
ASP3082	Heterobifunctional degrader (ternary complex with E3 ligase)	KRAS G12D protein (proteasomal degradation)	Preclinical; xenograft tumor regression reported	Yoshinari et al. 2025
BI-3406	SOS1-KRAS protein-protein interaction inhibitor	SOS1 nucleotide exchange factor (indirect KRAS targeting)	Preclinical; synergy with KRAS/MEK inhibitors	Hofmann et al. 2021
Sotorasib (AMG 510)	Covalent electrophile, switch II pocket	KRAS G12C (mutant cysteine)	FDA-approved (context/methodological precedent)	Canon et al. 2019; Hong et al. 2020
Adagrasib (MRTX849)	Covalent electrophile, switch II pocket	KRAS G12C (mutant cysteine)	FDA-approved (context/methodological precedent)	Fell et al. 2020
Virtual screening leads (e.g., Du et al. series)	Noncovalent, docking/MD-derived hits	Switch I/switch II pocket, KRAS G12D	Early computational hit-to-lead stage	Du et al. 2024; Issahaku et al. 2022
KRASAVA-prioritized candidates	QSAR/ML-prioritized noncovalent candidates	Switch I/switch II pocket, KRAS G12D	In-silico candidate proposal stage	Tinkov et al. 2026

Third, the emergence of degrader (ASP3082) and pathway-adjacent (SOS1 inhibitor) strategies alongside direct pocket-binding chemotypes suggests the field is treating KRAS G12D less as a single druggable-pocket problem and more as a portfolio problem, where different mechanisms of target engagement carry different resistance liabilities and different combination potential [18,19]. Computational methods will need to keep pace with that diversification — ternary complex modeling for degraders is a genuinely different structural prediction problem than single-protein pocket docking, and protein-protein interface targeting (as with SOS1) again calls on a somewhat different toolkit than small, buried allosteric pocket work. It would be a mistake to assume that the docking-MD-MMGBSA pipeline validated for switch I/II pocket binders transfers unmodified to these adjacent problems.

Finally, resistance is likely to become the dominant question for this class of compounds as clinical experience accumulates, much as it has for the G12C inhibitors. Computational prediction of resistance mutations — screening candidate resistance variants against existing inhibitor structures using the same MD and free energy tools reviewed here — is a natural extension of this methodology and one that several groups working on G12C resistance have already begun applying; it would be surprising if equivalent work on G12D-directed compounds does not follow shortly after broader clinical exposure to MRTX1133 and its successors.

Conclusion

KRAS G12D moved from "essentially undruggable" to "has an approved-track clinical candidate" within roughly a decade, and structure-based virtual screening, molecular dynamics simulation, and binding free energy calculation were central to that transition rather than incidental to it. The switch I/switch II allosteric pocket identified through this work gave medicinal chemists a genuine, structurally defined target; docking and pharmacophore-based virtual screening translated that structural insight into candidate chemical matter; molecular dynamics provided the conformational context needed to distinguish real binding modes from docking artifacts; and MM-GBSA and FEP-based free energy estimation supplied the quantitative ranking needed to prioritize compounds for synthesis. The resulting chemotypes — MRTX1133 and its close analogs, salt-bridge-forming noncovalent inhibitors, targeted degraders, and pathway-adjacent SOS1 inhibitors — now offer several independent routes to suppressing G12D-driven signaling, each with distinct resistance and combination profiles still being worked out. The next stage of progress will likely depend less on finding entirely new computational tricks and more on applying the existing toolkit — particularly rigorous free energy methods — earlier and more systematically in the discovery funnel, and on extending structural and dynamic modeling to the ternary-complex and protein-protein-interface problems that degrader and SOS1-directed strategies now present.

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